AMENDMENTS TO THE SPECIFICATION

In the specification

Delete from page 8, line 12, to page 10, line 13.

Amend the specification at page 1, lines 1-3 as follows:

This application is a ~~continuation-in-part of application Ser. No. 08/841, filed April 30, 1996, which was a~~ divisional application of application Ser. No. ~~08/841,751, filed April 30, 1996~~ 09/301,808, filed November 29, 1999~~, which was a divisional application of application Ser No. 08/609,572, filed March 1, 1996~~. The contents of this application are incorporated by reference in their entirety.

Amend the paragraph starting on page 29, line 19 as follows.

Specific binding was visualized under the microscope. Only cells transfected with IL-13 showed specific binding to IL-13bc-Ig. ~~(see photo of transfected cells, the Figure)~~.

Amend the paragraph starting on page 33, line 5 as follows.

To compare the regulatory roles of IL-4 and IL-13 in the pathogenesis of schistosomiasis, we infected C57BL/6 WT and IL-4 deficient mice percutaneously with 25 *S. mansoni* cercariae. Separate groups of animals were treated with either sIL-13Rα2-Fc or with control-Fc, as described in the Materials and Methods. The treatments began on week 5, at the start of egg laying, and all animals were sacrificed 8 wk postinfection and examined for several parasitologic and immunologic parameters. ~~As shown in Table III,~~ A~~a~~ll four groups of mice harbored similar worm burdens, and tissue eggs produced per worm pair did not vary among the groups. At 8 wk postinfection, the time of the peak tissue response[45], WT mice showed no significant change in granuloma size as a result of IL-13 blockade ~~(Fig. 2A)~~. Interestingly, control-Fc treated IL-4 deficient mice also failed to show reduced granulomatous response, and in fact, granulomas were significantly larger in these mice. In striking contrast to these observations, the IL-4 deficient mice displayed a markedly reduced granulomatous response when IL-13 was inhibited ~~(Fig. 2A, far right)~~. Indeed, the double IL-4 deficient/sIL-13Rα2-Fc-treated mice displayed on average a 40 to 50% reduction in granuloma volume when compared with either control or sIL-13Rα2-Fc-

treated WT animals, and more than a 75% reduction when compared with control-Fc-treated IL-4 deficient mice.

Amend the paragraph starting on page 33, line 23 as follows.

The cellular composition of the lesions was also evaluated in Giesma-stained liver sections and ~~as shown in Table III,~~ IL-4 deficient mice displayed a marked reduction in granuloma-associated mast cells. In contrast, there was no change in mast cell numbers by IL-13 inhibition alone, and IL-13 bloackade had no additional effect on the already highly reduced numbers of mast cells in IL-4-deficient mice. Somewhat similar, yet distinct findings were observed when granuloma-associated eosinophils were evaluated ~~(Fig. 2B)~~. Here, the numbers of eosinophils were increased from 46 to 64% in WT mice by IL-13 blockade and significantly decreased (28%) as consequence of IL-4 deficiency. Despite the apparent contrasting roles for IL-13 and IL-4 in the tissue eosinophilia, an evenmore striking combined inhibitory effect was observed when the IL-4-deficient mice were treated with IL-13 inhibitor. In these mice, the average number of granuloma eosinophils was below 10%. Finally, there was no change in the degree of parenchymal or egg-asociated liver necrosis in the WT versus IL-4-deficient animals, while both sIL-13Rα2-Fc treated WT and IL-4 deficient groups showed marked reductions in overall parenchymal necrosis.

Amend the paragraph starting on page 34, line 7 as follows.

Perhaps most importantly, the sIL-13Rα2-Fc treatment alone significantly reduced the collage content of liver granulomas in WT mice, as assessed in tissue sections staind with picrosirius red ~~(Table III and Fig. 3)~~. In contrast, infected IL-4-deficient mice showed no detectable change in granuloma collagen deposition by microscopic analysis. Interestingly, there appeared to be no combined or synergystic riole for IL-13 and IL-4 in this parameter since there was no significant difference between sIL-13Rα2-Fc-treated-WT and –IL-4 deficient mice ~~(Table III)~~. ~~Fig. 3 shows that w~~While similar worm numbers, tissue egg burdens, and granuloma sizes were found in control and sIL-13Rα2-Fc treated WT mice, IL-13 blockade had a substantial inhibitory effect on collagen deposition within the liver. Finally, the extent of hepatic fibrosis was also measured by the assessment of hydroxyproline levels ~~(Fig. 2C)~~, which is more quantitative than the histological techniques described above. The soluble IL-13 antagonist alone markedly decreased liver hydroxyproline levels, while the IL-4-deficiencyresulted in less significant reduction. The dual IL-4/IL-13 deficiency failed to reduce hydroxyproline to levels

below that already observed in the sIL-13Rα2-Fc treated WT mice ~~(Fig. 2C)~~, although there was a slight trend in a second study (not significant). Together, these data demonstrate that IL-13 is the dominant Th2-associated cytokine responsible for the development for the development of hepatic fibrosis in murine schistosomiasis.

Amend the paragraph starting on page 34, line 30 as follows.

While it is well-Known that IL-4 is the primary cytokine driving $CD4^+$ Th2 cell development [21, 22], the role of IL-13 in the generation and maintenance of Th2-type responses has been controversial and may be influenced by both host genetics and the infectious disease model under study [30,34,38]. Therefore, to determine whether the sIL-13Rα2-Fc-induced changes in liver pathology were generated by alterations in the Th1/Th2 cytokine balance, we isolated mesenteric lymph nodes and spleens from infected mice, prepared single cell suspensions, and restimulated the cultures *in vitro* with parasite antigens. Additional cell cultures were exposed to parasite antigens in the presence of anti-CD4 mAbs to determine whether cytokine production was dependent upon a $CD4^+$ T cell response. Culture supernatants were analyzed by ELISA for IL-4, IL-13, IL-5, IL-10, and IFN-γ. As might be predicted [15], mesenteric ~~(Fig. 5)~~ and splenic cultures (data not shown) prepared from WT mice displayed a highly polarized Th2-type cytokine response. They produce high levels of IL-4, IL-5, IL-10, and IL-13 in response to SEA stimulation and little or no IFN-γ. IL-4 deficient mice in constrast showed a more mixed Th1/Th2-type profile. Indeed, a significant SEA-specific IFN- γ response was detected in IL-4 deficient mice, which is consistent with previous studies [23,24]. IL-13, IL-10, and to a lesser extent IL-5, were also detected in these animals, although the levels of these cytokines were markedly decreased when compared with WT mice. Importantly, the maintenance of the low but significant IL-4-independent IL-13 response likely explains the marked granulomatous response that is maintained in the absence IL-4 ~~(Fig. 2)~~. Surprisingly, despite its marked inhibitory effect on hepatic fibrosis, sIL-13Rα2-Fc had no significant effect on Th1 or Th2-type cytokine responses in either WT or IL-4 deficient mice. It should also be noted that in all cases, cytokine production was highly dependent on a $CD4^+$ T cell response, since little or no cytokine expression was detected in any of the anti-CD4 mAb-treated SEA-stimulated cultures.

Amend the paragraph starting on page 35, line 29 as follows.

To determine whether a similar pattern of cytokine expression was observed *in vivo* at the site of granuloma formation, we isolated liver mRNA from the various groups of mice at 8 wk

postinfection and performed quantitative RT-PCR. ~~As shown in Figure 5, i~~Infected WT mice displayed a strong Th2-type cytokine mRNA profile, showing marked increases in IL-4, IL-13, IL-5, and IL-10 mRNA. The WT mice also showed modest increases in the expression of IFN-γ mRNA, which was consistent with previous observations [19]. In contrast to these findings, IL-13 and IL-5 mRNA levels were much lower in IL-4 deficient micem while IL-10 and TNF-α mRNA significantly increased and IFN-γ mRNA expression did not change. Again, similar to the in vitro results obtained from mesenteric lymph node and splenocyte cultures, IL-13 blockade had no significant effect on the pattern of cytokine mRNA expression in either WT or IL-4 deficient mice treated with the sIL-13Rα2-Fc, although this is unlikely to explain the decreases in fibrosis, since highly divergent levels of IL-10 were detected in sIL-13Rα2-Fc-treated WT versus IL-4 deficient mice, yet a similar decrease in fibrosis was observed. TGF-β1 and TGF-β2 mRNA expression was also examined in the granulomatous tissues, however no significant differences were observed in either infeced IL-4-deficient mice or in animals treated with sIL-13Rα2-Fc (data not shown).

Amend the paragraph starting on page 36, line 19 as follows.

The *in vitro* and *in vivo* cytokine studies decribed above suggested that the anti-fibrotic effect of sIL-13Rα2-Fc was unlikely to be explained by changes in Th1 or Th2-type cytokine expression. Therefore, in subsequent experiments, we investigated the patterns of collagen I (Col I) and collagen III (Col III) mRNA expression to determine whether the sIL-13Rα2-Fc-induced reduction in fibrosis was accompanied by direct changes in the expression of these two important collagen producing genes [19]. ~~As shown in Figure 6,~~ IL-13 blockade significantly reduced Col I and Col III mRNA expression in both WT and IL-4 deficient mice. There was no change in the infection-induced levels Col I or Col III mRNAs in IL-4-eficient mice and when compared with sIL-13Rα2-Fc-treated WT mice, there was no further reduction in similarly teated IL-4 deficient mice.

Amend the paragraph starting on page 37, line 1 as follows.

Having shown that IL-13 blockade *in vivo* significantly reduced Col I and Col III mRNA expression in the liver of infected WT and IL-4 deficient mice, we wanted to determine whether IL-13 would directly stimulate collagen synthesis in fibroblasts. To answer this question, we examined the induction of type I collagens in murine 3T3 fibroblasts by Western blotting. ~~As shown in Fig. 7,~~ IL-13 induced collagen synthesis 48 h after stimulation. Minimal type I

collagen was detected in unstimulated cells ~~(Fig 7, lane 1)~~ or at earlier time points in the cytokine-activated cultures (data not shown). IL-4 also induced collagen I synthesis ~~(lane 2)~~ and high levels of secreted collagen were easily detectable in the supernatants obtained from both cytokine-stimulated cultures (data not shown). The specificity of the reaction was confirmed by using purified collagen type I ~~(lane 5)~~ and bacterial collagenase treatments showed that the antibodies were specific for collagen (data not shown).

Amend the paragraph starting on page 37, line 31 as follows.

Several studies have shown that Th2-type cytokine responses can develop *in vivo* in the absence of IL-4 or the IL-4 receptor [26, 39], which is consistent with our findings since reduced but significant IL-13, IL-10, and IL-5 expression was detectd in the mesenteric lymph nodes ~~(Fig. 4)~~ and livers ~~(Fig. 5)~~ of infected IL-4-deficient mice. Their production was also highly dependent on a $CD4^+$ T cell response ~~(Fig.4)~~, further indicating that a conventional Th2-type response was established. These findings provide evidence that while maximal IL-13 expression is dependent on IL-4, the continued production of IL-13 might explain the maintenance of a significant granulomatous response in the absence of IL-4 [23-25]. Ideed, while blocking IL-13 alone had no effect on granuloma size in WT mice, inhibiting the residual IL-13 in IL-4-deficient mice resulted in a marked and highly significant reduction in granuloma volume ~~(Fig. 2A)~~. These findings demonstrate that IL-4 and IL-13 are both sufficient to mediate granuloma development, and formally explain the production of granulomas in IL-4-deficient mice versus the nearly complete lack of granulomas in Stat6-deficient mice [16,24]. They also support recent findings in the pulmonary egg granuloma model [30]. Because granulomas serve an important host-protective role by walling off potentially lethal hepatotxins released by the eggs [47], the host may have evolved redundant mechanisms for granuloma formation in order to ensure a favorable host-parasite relationship.

Amend the paragraph starting on page 38, line 19 as follows.

While these observations clearly demonstrate that IL-4 and IL-13 actively participate in granuloma formation, unique roles for both cytokines in mast cell recruitment, tissue eosinophilia, and most importantly, the generation of hepatic fibrosis were revealed in these studies. Histological examinations of liver sections from infected mice demonstrated that IL-13 is not required for mast cell ~~(Table III)~~ or eosinophil ~~(Fig. 2B)~~ differentiation and recruitment, since granulomas of sIL-13Rα2-Fc-treated WT mice showed no decrease in either cell type. In

fact, eosinophil numbers were significantly increased in the lesions of IL-13-inhibited WT mice ~~(Fig. 2B)~~, suggesting that IL-13 may partially antagonize this effect. In contrast, mast cells were almost completely absent from the lesions in IL-4-deficient mice and eosinophils were decreased by over 50%. Interestingly, IL-13 appears to partially support the reduced but significant egg-induced tissue eosinophilia in IL-4-deficient mice since eosinophils were reduced to belo 10% in the IL-4-deficient/sIL-13Rα2-Fc-treated animals. Nevertheless, these data indicate that IL-4 is the dominant cytokine responsible for the development of eosinophil and mast cell populations within granulomas.

Amend the paragraph starting on page 39, line 3 as follows.

Probably the most important advance from this study was the finding that hepatic fibrosis could be blocked y sIL-13Rα2-Fc. Indeed, microscopic ~~(Table III)~~, biochemical ~~(Fig. 2C)~~, and molecular techniques ~~(Fig.6)~~ all indicated that IL-13, not IL-4, plays the major role in the development of egg-induced liver fibrosis. Previous studies showed that the Th1/Th2 cytokine balance can significantly effect the extent of tissue fibrosis in *S. mansoni* infected mice [19]. Nevertheless, this study suggests that the effects of sIL-13Rα2-Fc were not mediated through a skewing of the Th cell cytokine response. Blocking IL-13 had no significant effect on the production of IFN-$_y$, IL-4, IL-5, IL-10, or IL-13 by mesenteric lymph node ~~(Fig. 4)~~ or spleen cells *in vitro* and there was also no change in cytokine mRNA expression *in vivo*, at the site of lesion formation ~~(Fig. 5)~~. In contrast to these observations, IL-4-deficient mice displayed an increase IFN-$_y$ response in the draining lymph nodes ~~(FIG. 4)~~ and decreased IL-5 and IL-13 expression in both the lymph nodes ~~(Fig.4)~~ and live ~~(Fig. 5)~~. Thus, the slight reduction in fibrosis detected in IL-4-deficient mice by hydroxyproline analysis ~~(Fig. 2C)~~ may be attributable to decreased IL-13 production. The fact that IL-4 production was unaffected by IL-13 blockade, yet fibrosis was maximally reduced in these animals emphasizes the important role played by IL-13. Indeed, sIL-13Rα2-Fc-treated IL-4 deficient mice showed little additional decrease in hydroxyproline levels ~~(Fig. 2C)~~ and no difference in Collagen I or III mRNA expression ~~(Fig. 6)~~ over that observed in similarly-treated WT mice. There was also no change in Collagen I or III expression in control-Fc-treated IL-4-deficinet mice when compared with WT anmials, further de-emphasizing the contribution of IL-4. Moreover, *in vitro* studies with 3T3 cells demonstreated for the first time the ability of UL-13 to stimulate collagen production in fibroblasts ~~(Fig.7)~~, thus the effects of IL-13 on fibrosis may be more direct and not dependent

upon modulations in the Th1/Th2 cytokine response. In support of this conclusion, recent studies demonstrated that IL-13 receptors are expressed on fibroblasts [32] and that IL-13 increase adhesion molecule and inflammatory cytokine expression in human lung fibroblasts [48]. Finally, although IL-13 ~~(Fig. 7)~~ and IL-4[49] are both capable of promoting collage production in fibroblasts, the fact that cultured lymph node cells produced nearly 100-fold more IL-13 than IL-4 ~~(Fig. 4)~~, only serves to emphasize the potentially important contribution of IL-13 in this process. Indeed, studies in the pulmonary granuloma model revealed that IL-4 mRNA expression is more tightly regulated at the site of lesion formation, while the induction of IL-13mRNA is much more sustained over the [30]. Nevertheless, we have not examined the kinetics of IL-4 and IL-13 mRNA expression in infected animals, so we can not say whether a similar pattern holds in the granulomatous livers.